Experimental Feline Viral Rhinotracheitis in the Germfree Cat

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FELINE RHINOTRACHEITIS VIRUS (feline herpesvirus) is generally considered the most important cause of respiratory disease in the domestic cat.[1-9] Susceptible cats inoculated intranasally with feline rhinotracheitis virus (FRV) develop acute upper respiratory disease characterized by sneezing, coughing, pyrexia, leucocytosis, mucopurulent rhinotracheitis, conjunctivitis, and tonsillitis.[9] Early in the course of the disease, eosinophilic, intranuclear inclusion bodies are associated with focal necrosis of upper respiratory epithelium. Neutrophilic infiltration and mucosal ulceration rapidly follow, presumably as a result of secondary bacterial infection.[9] The respiratory microflora, therefore, appears to play an important role in the pathogenesis of feline viral rhinotracheitis.

Distinguishing the effects of the primary pathogen from those of secondary invaders is a problem commonly encountered in the study of infectious diseases. In viral respiratory and gastrointestinal infections the role of the microbial flora is particularly difficult to determine. The objective of this study was to characterize the clinical disease and lesions produced by FRV in the absence of a microbial flora by experimentally infecting germfree cats.

Materials and Methods

The germfree cats were obtained by hysterectomy of pregnant queens from the specific-pathogen-free (SPF) colony of cats maintained in this department. All cats in the breeding colony are of caesarian-derived, colostrum-deprived ancestry and are maintained in complete isolation from other animals. The techniques for raising germfree cats have been described.[10]

Sterility was determined by culturing swabs of placenta and amniotic fluid at the time of surgery, and swabs of feces, urine, nasopharyngeal fluids, water, diet, and isolator surfaces at weekly intervals. Culture media consisted of thioglycolate broth, tryptose agar plus 5% equine blood, and PPLO agar. Inoculated media were incubated at 22, 37, and 55°C in aerobic and anaerobic environments. At necropsy aliquots of lung, spleen, and colon were cultured as above for bacteria and myco-

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plasma. The absence of indigenous cytopathic feline viruses was confirmed by inoculation of primary feline kidney cell cultures with 10% suspensions of spleen, lung, and fluid expressed from nasal and pharyngeal swabs.

The prototype C-27 strain of FRV (obtained from Dr. J. H. Gillespie, New York State Veterinary College, Ithaca, NY) had been purified by three plaquings and propagated in a feline diploid tongue cell culture system known to be free of mycoplasma.[11] The infective cell culture fluid, having a titer of 1×10^8 $TCID_{50}$/ml in primary feline kidney cell cultures, was stored at −190°C until use.

Thirteen 8-week-old germfree cats, representing 5 litters, were used in this investigation. Eleven were inoculated intranasally with 1×10^8 $TCID_{50}$ of virus contained in 1.0 ml of cell culture fluid. Two littermate control cats were inoculated with 1.0 ml of fluid from uninoculated cell cultures and housed in separate isolators. For 2 days prior to inoculation and throughout the course of the experiment, rectal temperatures of all cats were recorded 5 times each day at 4-hr intervals. Physical examinations were performed daily and food and water consumption recorded. Serum was collected from all germfree cats before inoculation and at necropsy and from the dams of all germfree litters.

Cats were killed by injection of sodium pentobarbital at intervals representing the various stages of disease from postinoculation days (PID) 2–31. Two of the infected germfree cats died on PID 5 and 6, respectively. Representative portions of all organ systems were fixed in Bouin's fluid, sectioned at 6 μ, stained with hematoxylin and eosin, and examined microscopically. Selected sections of turbinate were stained with periodic acid-Schiff, Giemsa, Gomori's trichrome, and Wilder's reticulin stain.

Ten percent suspensions of spleen, lung (left apical lobe), brain (temporal lobe), colon, and swabs of the pharynx, nostrils, and conjunctival sac were collected and frozen at −60°C for viral reisolation in primary feline kidney cell cultures. Each of 4 monolayers was inoculated with 0.2 ml of tissue suspensions, blood, or fluid in which swabs had been expressed and incubated for 2 hr at 37°C before addition of maintenance medium. Tubes were observed for cytopathic effect for 14 days after inoculation. The presence of FRV was determined by its characteristic cytopathic effect and the presence of eosinophilic intranuclear inclusion bodies in coverslips stained with hematoxylin and eosin.

Serum neutralization tests were performed in primary feline kidney cell cultures using serial twofold dilutions of serum which had been inactivated at 56°C for 30 min. One hundred $TCID_{50}$ of virus was added to an equal volume of serum and incubated at room temperature for 1 hr. Five monolayer cultures were inoculated with 0.2 ml of the serum/virus mixture and incubated at 37°C for 2 hr. The cultures were observed for cytopathic effect over a 14-day period and the 50% serum neutralizing titers calculated by the method of Reed and Muench.[12]

Results

Clinical Signs

All germfree cats inoculated with FRV developed severe upper respiratory disease characterized by epiphora, paroxysmal sneezing and coughing, dyspnea, listlessness, anorexia, odynophagia, and polydipsia (Table 1). Initially, nasal and conjunctival exudate was serous but became viscid and opalescent from PID 5 through 9 when dyspnea was most pronounced (Fig 1). Two cats developed subnormal temperatures and died on PID 5 and 6. Weight loss approximated 20% of preinoculation

Table 1. Clinical Signs Correlated with Histologic Changes in Nasal Mucosa, Virus in Nasal Fluids, and Serum Neutralizing Antibody in Germfree Cats Inoculated Intranasally with Feline Herpesvirus

	Days Postinoculation					
	1–2	3–4	5–6	7–10	13	31
Signs						
Fever	−	+	−	−	−	−
Sneezing and coughing	++	+	−	−	−	−
Nasal exudate	+	++	+++	+++	+	−
Dyspnea	+	++	+++	++	−	−
Decreased food consumption	+	+++	+++	+++	−	−
Increased water consumption	+	+++	+++	+++	−	−
Listlessness	+	++	++	+++	−	−
Weight loss	−	+	++	+++	+	−
Death	−	−	2/11	−	−	−
Nasal lesions						
Intranuclear inclusions	++	++	+	−	−	−
Epithelial necrosis	+	++	+++	+++	−	−
Neutrophil infiltration	−	++	++	−	−	−
Mononuclear cell infiltration	−	−	++	+++	+	+
Epithelial regeneration	−	−	−	+	++	+++
Virus in nasal fluids	+	+	+	+	+	−*
Serum neutralizing antibody	−	−	−	−	−	+

Minus sign (−) indicates absent; +, mild; ++, moderate; and +++, severe or extensive.

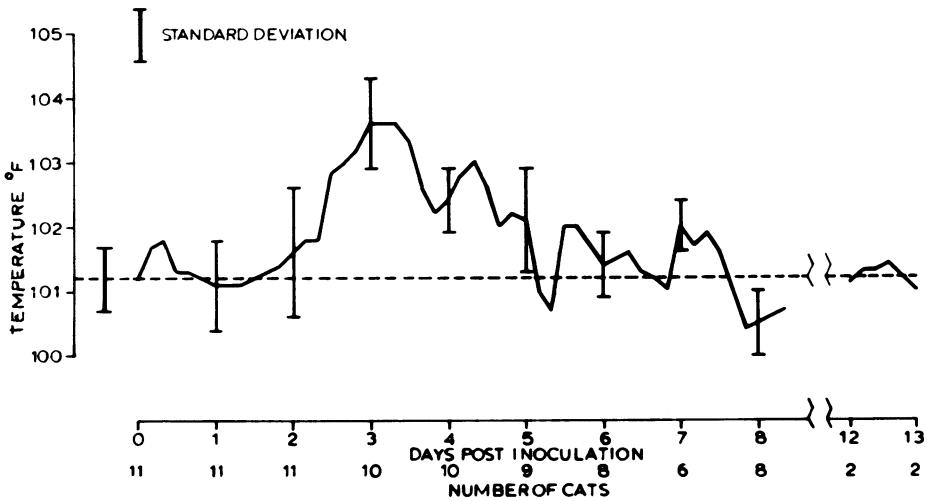
* Virus isolated from pharyngeal and nasal swabs as late as PID 20.

weight by PID 9. Clinical recovery began on PID 11 and was rapid thereafter.

Fever occurred on PID 3 and 4 (Text-fig 1). The peak mean rectal temperature was 103.6°F 72 hr PI. The temperatures of the infected cats returned to and remained within the preinoculation baseline range after PID 4. Hematologic exam showed leucocytosis, neutrophilia, and lymphopenia (Text-fig 2). The number of band neutrophils in infected cats increased 2–15 times above preinoculation mean 250 ± 105 cells/cu mm. Total erythrocyte counts, hemoglobin concentration, and packed cell volume were not altered significantly during the disease.

Gross Lesions

The nasal mucosa of cats killed from PID 6 through 9 was thickened and whitish, blending with the viscid, grey-white exudate which filled the nasal passages and frequently making the outlines of turbinate scrolls difficult to discern (Fig 2). Other lesions in cats killed from PID 6 through



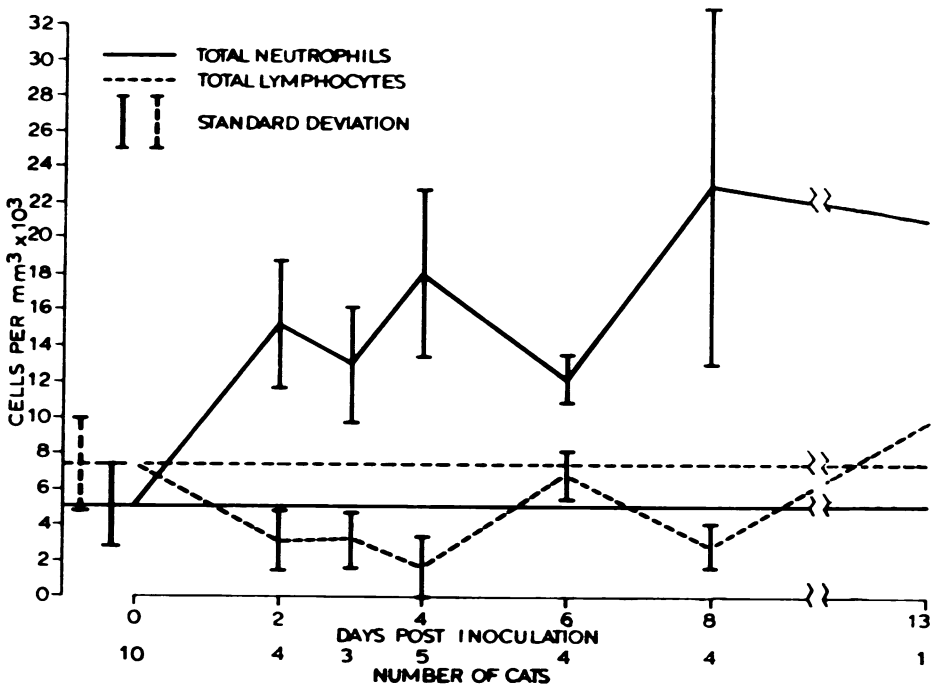
TEXT-FIG 1. Mean rectal temperatures of 11 germfree cats inoculated intranasally with feline herpesvirus illustrating fever on PID 3 and 4. *Broken horizontal line* represents mean preinoculation temperature of all cats.

13 included: congestion of the proximal trachea, occasional small areas of atelectasis in the apical and/or cardiac lobes of lungs, and moderate enlargement of the mandibular and pharyngeal lymph nodes. Lesions were not observed in the control germfree cats.

Microscopic Lesions

Nasal Mucosa and Turbinates. Multifocal epithelial lesions characterized by disorientation and discontinuity of cells, clumping and margination of chromatin, eosinophilic intranuclear inclusion bodies, and cytoplasmic hydropic degeneration were present in the nasal mucosa on PID 2 (Fig 3 and 4). Epithelial necrosis progressed rapidly and was accompanied by neutrophilic infiltration. Most of the mucosal epithelium of the nasal, maxillary and ethmoid turbinates, nasal septum, frontal, maxillary and sphenoidal recesses, and nasopharynx was necrotic by PID 5 (Fig 5). Many of the mucosal tubuloalveolar glands, capillaries, and venules were destroyed while arteries and veins in the deeper portions of the mucosa were rarely necrotic. The olfactory mucosa was represented chiefly by olfactory nerve fasciculi and portions of Bowman's glands surrounded by necrotic cellular debris.

The nasal passages became filled with necrotic epithelial cells, neutrophils, macrophages, and fibrin by PID 6. The underlying nasal mucosa was densely infiltrated by macrophages, lymphocytes, and a few plasma cells and eosinophils. A layer of squamous epithelium had partially re-



TEXT-FIG 2. Mean total neutrophil and lymphocyte counts of germfree cats inoculated intranasally with feline herpesvirus illustrating neutrophilia and lymphopenia. *Horizontal lines* represent preinoculation mean counts of all cats.

generated over the denuded, densely infiltrated mucosa by PID 8 (Fig 6). On PID 13 the nasal mucosa was edematous, contained scattered aggregates of mononuclear leukocytes, and was covered by squamous epithelium which also proliferated over strands of fibrinous exudate still attached to the mucosa resulting in epithelial adhesions or tags of epithelium trailing into the nasal passages (Fig 7). No inclusion bodies or evidence of continuing epithelial necrosis were present in the few surviving segments of nasal epithelium which remained.

Regeneration of mucociliary but not olfactory epithelium was evident on PID 31. Degenerating olfactory nerve fasciculi were present in the ethmoturbinals, cribriform plate, and on the surface of the olfactory bulbs. Scattered infiltrations of lymphocytes and plasma cells were present throughout the nasal mucosa and within the olfactory bulbs.

Resorption of bone and increased numbers of osteoclasts were evident in the turbinates of the infected cats killed after PID 4. In 4 cats there was severe lysis of portions of the maxillary turbinate bone in addition to necrosis of the overlying nasal mucosa. The affected turbinates were represented only by interrupted fragments of bone surrounded by cell-

ular debris and scattered osteoclasts (Fig 5). Such severe necrotizing lesions were suggestive of ischemic coagulation necrosis. Inclusion bodies could not be demonstrated in the nuclei of osteoblasts, osteocytes, or endothelial cells.

Extranasal Lesions. Focal necrotizing lesions were present in the mucosal epithelium of the tonsils of 6 cats, pharynx of 1 cat, conjunctiva of 7 cats, and epiglottis of 6 cats (Table 2). Inclusion bodies were demonstrable through PID 6. Focal lymphocytic infiltrations were associated with healing epithelial lesions in cats killed at later intervals.

Laryngeal and tracheal lesions characterized focal epithelial disorientation, depletion of mucus from goblet cells, nuclear pyknosis, and absence of cilia; desquamation of cells was observed in 8 cats (Table 1). Inclusion bodies were infrequent and never was more than one-third of the tracheal epithelium in any section destroyed. Lesions were more frequent in the anterior trachea than in the midcervical or thoracic regions.

Four cats had focal lesions in the bronchi or bronchioles of the apical or cardiac lobes of the lung. Epithelial necrosis with eosinophilic intranuclear inclusion bodies was present in 2 cats while the remaining 2 had regions of metaplastic epithelium associated with subjacent mononuclear cell infiltration.

Table 2. Distribution of Lesions in Germfree Cats Inoculated Intranasally with Feline Herpesvirus

		Lesions							
Cat No.	Days post-inoculation	Nasal mucosa	Turbinate bone*	Tonsillar epithelium	Pharynx	Conjunctiva	Epiglottis	Larynx/Trachea	Bronchi/Bronchioles
1	2	+	−	−	−	−	−	−	−
2	2	+	−	+	−	−	−	+	−
3	4	+	−	−	−	−	−	+	−
4	5	+	+	+	+	−	+	+	−
5	6	+	+	+	−	+	+	+	−
6	6	+	−	+	−	+	+	+	−
7	8	+	−	+	−	+	+	−	+
8	9	+	+	+	−	+	+	+	+
9	9	+	+	−	−	+	+	+	+
10	13	+	−	−	−	+	+	+	−
11	31	+	−	−	−	+	+	−	+
12	Control	−	−	−	−	−	−	−	−
13	Control	−	−	−	−	−	−	−	−

(+) indicates lesions observed; −, no lesions observed.
* Severe lysis of bone.

Lymphoid Tissues. The spleen and lymph nodes of control germfree cats generally contained only primary follicles of small lymphocytes. Reticuloendothelial cells were inconspicuous. The most conspicuous histologic changes in the lymphoid tissues of the infected cats occurred in the mandibular and pharyngeal lymph nodes. Edema, moderate depletion of small lymphocytes, and mild reticuloendothelial hyperplasia were evident on PID 5. From PID 8 to 13, the cortex and medulla became filled with a mixture of lymphoblasts, reticuloendothelial cells, and small lymphocytes. A few rudimentary reactive follicles were present in the cortex on PID 13 and distinct reactive follicles were evident on PID 31.

Histologic changes in the spleen and bronchial lymph nodes were similar to but milder than those described above. Depletion of lymphocytes with little associated reticuloendothelial hyperplasia was evident in the thymus of cats necropsied from PID 5 through 13. No lesions were observed in other tissues of the infected cats or in the control cats.

Virus Reisolation

FRV was isolated from all nasal, pharyngeal, and conjunctival swabs taken from PID 2 through 20. No virus could be recovered from the swabs collected on PID 31. The virus was recovered from suspensions of lung from all cats necropsied between PID 5 and 9 but not from the lungs of cats killed before or after this period. The virus was also isolated from the blood of 1 cat killed on PID 2 but not from the spleen of the same cat. No virus could be detected in blood from 3 other cats on PID 2 or from 20 other blood samples taken from PID 1 through 13.

In none of the spleen, brain, or colon suspensions of infected germfree cats was FRV or any other cytopathic agent detected. No cytopathic agents were detected in the nasal and pharyngeal fluids, lung, spleen, brain, or feces of the control germfree cats.

Neutralizing Antibody

No neutralizing activity could be demonstrated in the pre- or postinoculation serums of: (1) infected germfree cats killed from PID 2 to 13, (2) control germfree cats, or (3) SPF dams of the germfree litters. The serum of the cat killed on PID 31 had a 50% neutralizing antibody titer of 1:10.

Sterility

No bacteria or mycoplasmas were detected in the food, water, nasopharyngeal fluids, urine, feces, colon, spleen, or lung of either infected or control cats using the established techniques described.

Discussion

Feline viral rhinotracheitis was a surprisingly severe disease in germfree cats. The pathogenicity of FRV is apparently not dependent upon the synergistic activity of the respiratory microbial flora. Clinically and histologically, experimental feline rhinotracheitis in germfree cats was similar to the disease in conventional cats.[9] The ulcerative, fibrinopurulent rhinitis in conventional cats had been attributed chiefly to the activity of secondary bacterial invaders. In germfree cats, however, the virus rapidly destroyed virtually all intranasal epithelium. The initial neutrophilic inflammatory response which ensued was apparently elicited by products of epithelial necrosis. To our knowledge the lesions produced by FRV are the most severe of any viral disease reported thus far in germfree animals. By contrast, the respiratory and gastrointestinal lesions which characterize viral diseases such as canine distemper and feline infectious enteritis are completely absent in germfree animals.[13,14]

The distribution of lesions produced by FRV in germfree and in conventional cats is identical, with two exceptions: (1) the bronchial and bronchiolar lesions, which occurred in germfree cats have not been previously reported, (2) no lesions were observed in the lingual or oral mucosa of germfree cats suggesting that the ulcerative glossitis which sometimes accompanies viral rhinotracheitis in conventional cats [7] may not be caused by FRV. Despite the severe nasal lesions and abundance of virus in nasal and pharyngeal fluids, laryngotracheal lesions were mild and pulmonary lesions confined to bronchi and bronchioles.

Resorption of turbinate bone has been described in experimental feline rhinotracheitis in conventional cats [9] but osteolytic lesions simulating overt necrosis of bone were not reported. It was not possible to determine whether the osteolytic lesions in germfree cats were due to (1) osteolysis provoked by the severe necrotizing rhinitis, (2) ischemic necrosis secondary to vascular lesions, or (3) viral infection of osteogenic cells with subsequent necrosis of bone. Preliminary studies indicate that FRV inoculated intravenously into susceptible 8-week-old cats, produces osteolytic lesions in the turbinates which are not preceded by necrosis of nasal epithelium.[15] Focal necrotizing lesions also occur in the primary spongiosa of the ribs and long bones. Reports relating viruses to lesions in bone are rare. Only the H-viruses and Rat virus have thus far been shown to selectively affect bone.[16] The apparent alteration in pathogenesis of infection following intravenous inoculation and the tropism of the virus for growing bone are presently under investigation.

Considering the widespread destruction of olfactory epithelium and the affinity of many herpesviruses for the central nervous system, it is

noteworthy that encephalitis did not occur in germfree cats inoculated with FRV. The only lesions referable to the central nervous system were degeneration of olfactory nerve fibers and focal lymphocytic infiltration in the olfactory bulbs.

Resolution and repair of the lesions in the upper respiratory mucosa occurred while virus could still be isolated from nasal fluids and before detectable levels of neutralizing antibody were present in the serum. The cessation of epithelial necrosis and the onset of epithelial regeneration correlated well with the occurrence of lymphocytic infiltration in the nasal mucosa. The relatively meager serologic response following infection with FRV has been observed previously.[9,17] Some convalescent cats, however, are apparently resistant to reinfection although little or no serum antibody is detectable.[17] Perhaps either interferons or locally produced antibody similar to IgA in certain human upper respiratory infections [18] are important in recovery and resistance in feline viral rhinotracheitis.

Summary

Germfree cats inoculated intranasally with feline rhinotracheitis virus (FRV) developed severe upper respiratory disease. Clinically, disease was characterized by fever, neutrophilic leucocytosis, paroxysmal sneezing and coughing, copious nasal exudate, dyspnea, anorexia, and pronounced weight loss. Histologically, eosinophilic intranuclear inclusion bodies were associated with extensive, necrotizing rhinitis and focal epithelial necrosis in the conjunctiva, tonsils, epiglottis, larynx, trachea, and occasional bronchi and bronchioles. FRV was reisolated from the nasal and pharyngeal fluids from post inoculation day (PID) 2 through 20 and from the lungs between PID 5 and 9. The virus was reisolated from the blood of 1 cat killed on PID 2 but not from the spleen, brain, or colon of any cats. No neutralizing antibody was detected in the serums of cats killed from PID 2 to 13. The serum of 1 cat killed on PID 31 had a neutralizing antibody titer of 1:10/100 $TCID_{50}$ of virus.

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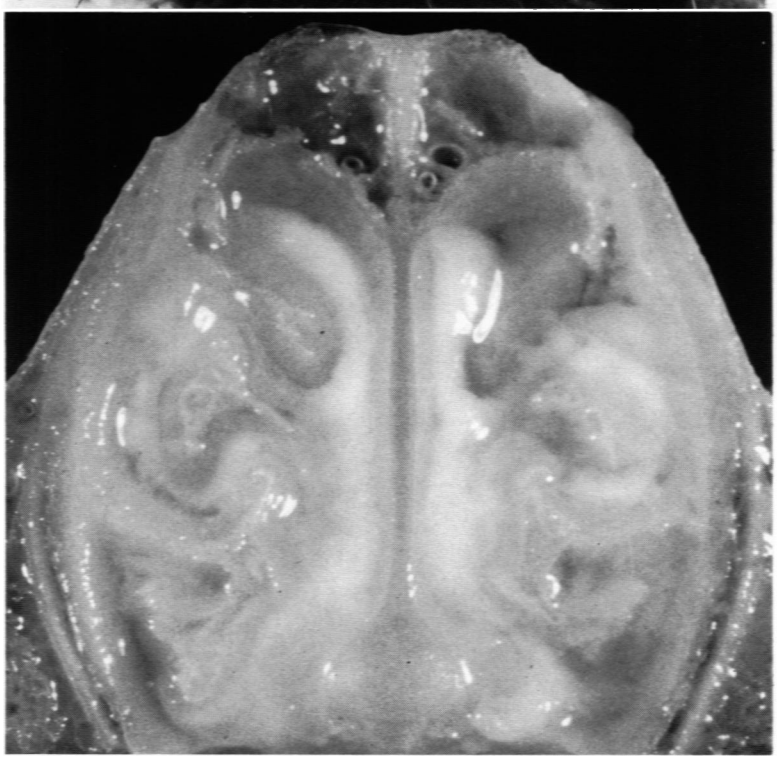

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Fig 1. Germfree cat—7 days after intranasal inoculation with feline rhinotracheitis virus. Viscid, opalescent nasal, and conjunctival exudate is evident at nostrils and on facial hair.

Fig 2. Cross-section of anterior nasal fossa of germfree cat—7 days after inoculation with feline rhinotracheitis virus. Thickened nasal mucosa blends with viscid, grey-white exudate fills nasal passages making profiles of turbinate scrolls difficult to discern.

Fig 3. Eosinophilic intranuclear inclusion bodies *(arrows)* and cytoplasmic hydropic degeneration in nasal epithelium of germfree cat—2 days after intranasal inoculation with feline rhinotracheitis virus. H & E. × 1250.

Fig 4. Degeneration and sloughing nasal epithelium of germfree cat—4 days after intranasal inoculation with feline rhinotracheitis virus. H & E. × 1250.

Fig 5. Turbinate of germfree cat—6 days after intranasal inoculation with feline rhinotracheitis virus. Massive necrosis of mucosal epithelium and severe lysis of turbinate bone, represented by fragments of bone (*B*) and cartilage (*C*) surrounded by necrotic cellular debris. H & E. × 125.

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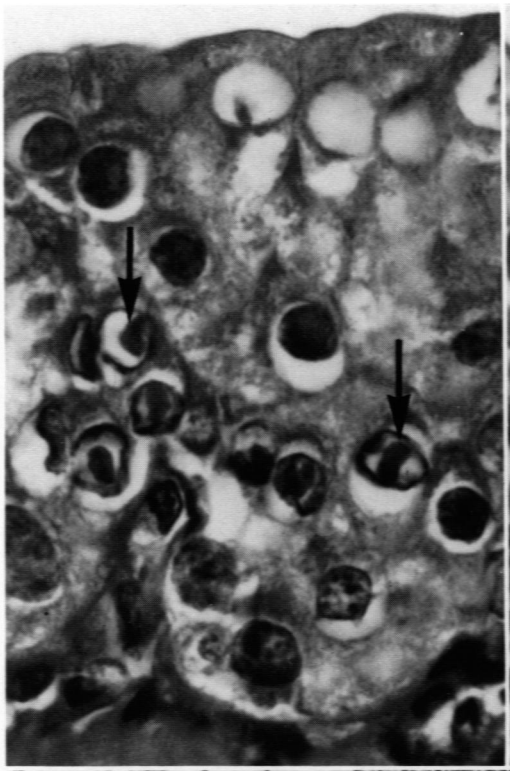

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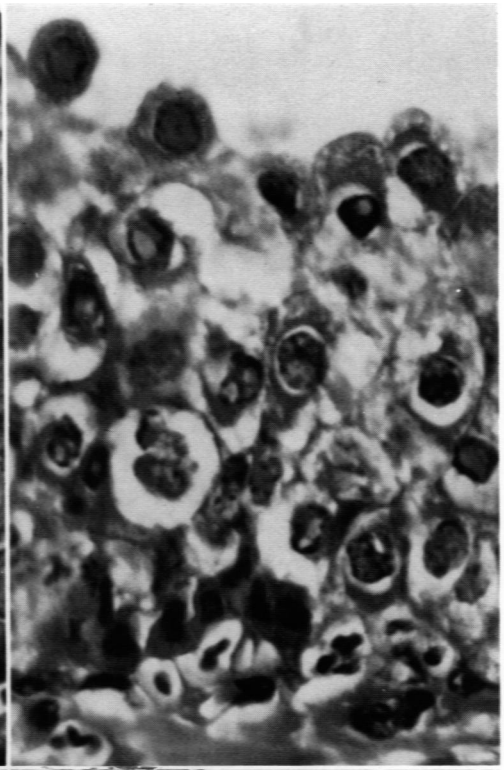

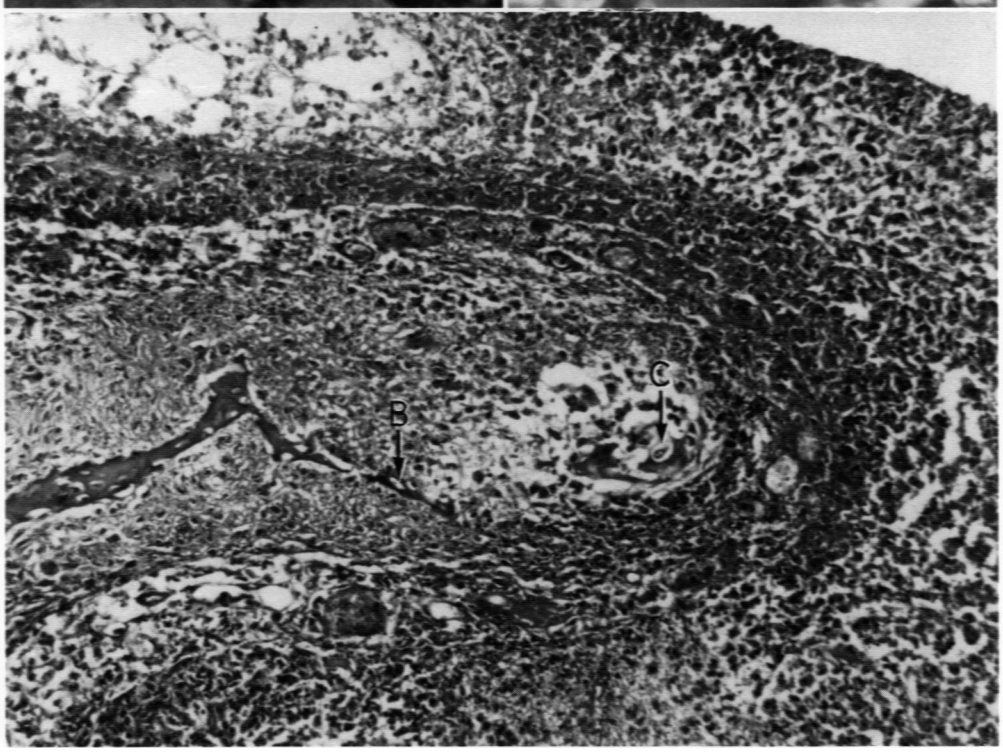

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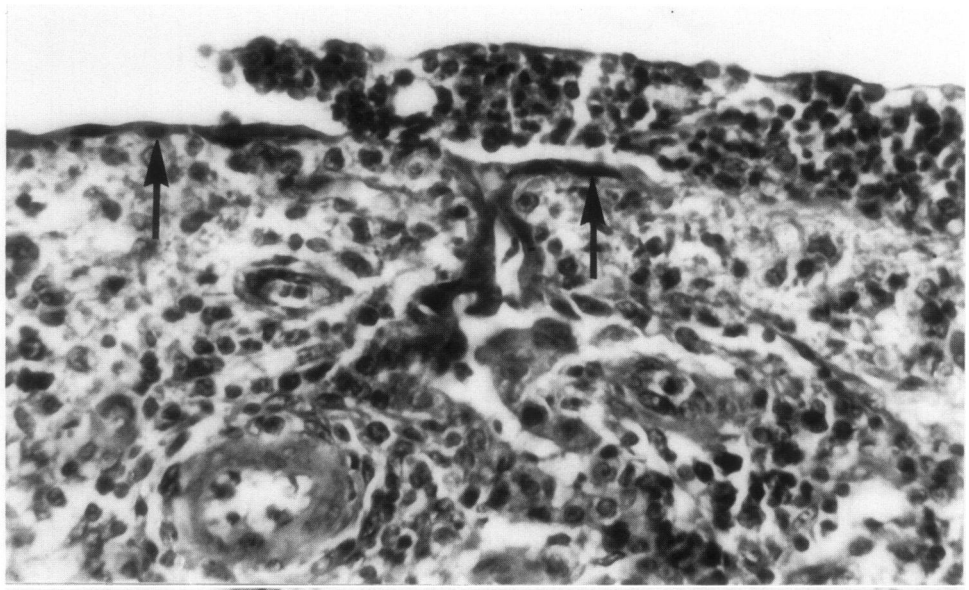

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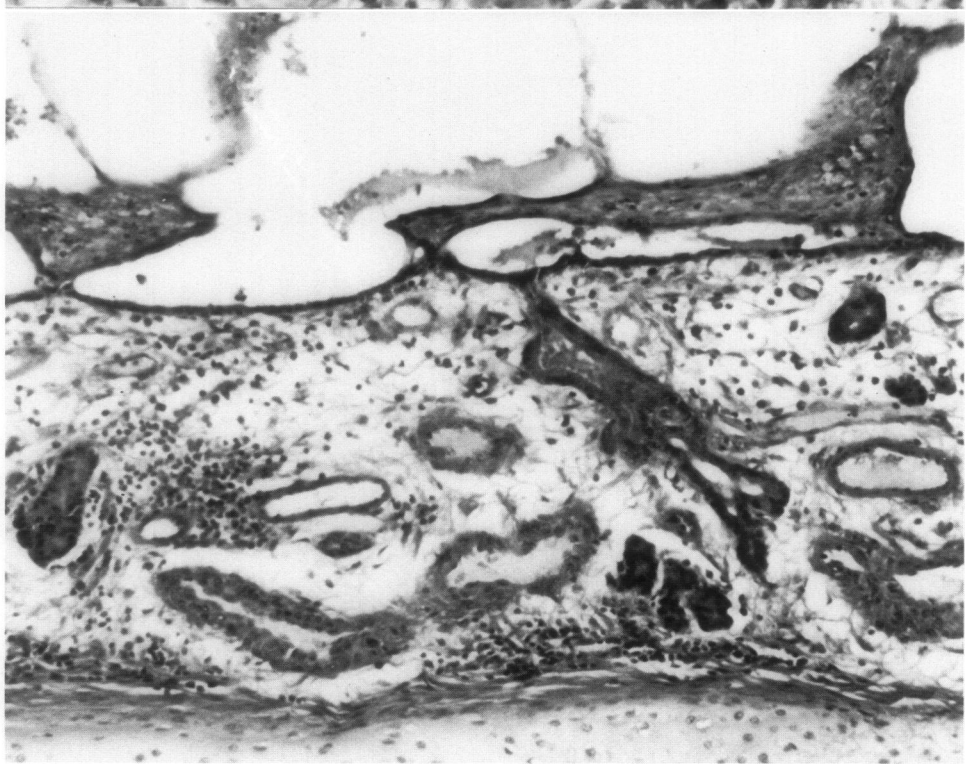

Fig 6. Nasal mucosa of germfree cat—8 days after intranasal inoculation with feline rhinotracheitis virus. Most necrotic cellular debris has sloughed into nasal passages, and metaplastic squamous epithelium *(arrow)* is regenerating over denuded mucosa. H & E. × 315.

Fig 7. Nasal mucosa of germfree cat 13 days after intranasal inoculation with feline rhinotracheitis virus. Layer of regenerative squamous epithelium covers mucosa as well as some tags of fibrinous exudate attached to mucosa. Mucosa is edematous and contains scattered lymphocytic infiltrations. H & E. × 125.